

**ATTACHMENT A****SUBSTITUTE SPECIFICATION**

(Including All Changes Made to the Specification and Abstract in International Application No. PCT/EP2005/000206)

**FORMULATION FOR TREATING OBESITY AND ASSOCIATED METABOLIC SYNDROME****BACKGROUND OF THE INVENTION****FIELD OF THE INVENTION**

[0001] The present invention relates to a formulation for treating obesity and associated metabolic syndrome

**DESCRIPTION OF THE RELATED ART**

[0002] Obesity is increasing at an alarming rate in most countries. It is now well recognized that this development dramatically increases the incidence of type 2 diabetes - and to some extent cardiovascular disease, hypertension, and cancer - known as metabolic syndrome and contributing to the reduction of life expectancy by several years. During the second half of the 20th century the rate of obesity has increased 5-10 fold and includes countries of the Far East and Asia, which previously suffered from undernutrition as the biggest problem. The epidemic of obesity is progressing very rapidly and constitutes the biggest health problem of the modern world

[0003] Many of the available methods of treating obesity cause only a temporary loss of weight, and after the termination of dietary treatment patients face a rapid weight regain. Slimming therapies involve primarily increased activity - physical exercises and reduced intake of calories. Discontinuation of the diet is associated with

a regain of body weight and the so called yo-yo effect. Increasing difficulties, along with continuation of weight reducing programs based on calorie restriction and rapid weight regain, are considered to be caused by the decrease of metabolic rate and thermogenesis. The decrease depends on the adaptation of the body to the changed energy balance and decreased food intake. Adaptation to low calorie intake includes, among other effects, decreased activity of the sympathetic nervous system and changes in the metabolism of thyroid hormones - towards production of the less active forms of the those hormones. Decreased activity of the adrenergic nervous system and a changed concentration of the active form of thyroid hormones lead to decreased mobilization and oxidation of fatty acids, and the decreased activity expression of uncoupling proteins UCP 1-3. The decrease of metabolic rate is one of the main reasons for the low rate of success of obesity treatment with more than 90% of the slimming patients returning to their starting weight.

[0004] In view of these known facts it is not surprising to find that other methods of treating obesity involve efforts to increase the metabolic rate. Calories stored in the body, mainly in a form of fat, are burned quicker due to administration of various thermogenetic drugs, which results in loss of body weight. However, those therapies often have negative side effects and, again, even here after discontinuation of the therapy the patient's body weight goes back to the starting value before treatment.

[0005] Different types of vegetable extracts are often used as adjuncts in the dietary treatment of obesity. One of the agents recently used is Green tea extract. Green tea, (*Camellia sinensis*) includes a large amount of catechin polyphenols, mainly epigallocatechin gallate - EGCG. Epigallocatechin gallate is a very strong

antioxidant, and it also reduces the appetite ,which leads to a decrease of food intake (see Am. J. Clin. Nutr 2000, 72, pages 1232-1234). Green tea extracts have been described to inhibit carbohydrates and lipids digestion and to exhibit strong anti-inflammatory activity. The thermogenetic effect of Green tea activity has been described in Am. J. Clin. Nutr. 1999, 70, pages 1040-1045. The authors reported that an extract rich in polyphenols and caffeine effectively and significantly increases energy expenditure and fat oxidation.

**[0006]** Another very popular therapeutic plant is an herb that grows in South America and called Yerba Maté (Paraguay, *Ilex paraguariensis*), containing triterpenes, caffeine, and caffeine-like compounds. Throughout America *Ilex paraguariensis* is used in a form of teas and infusions even more often than coffee. In Germany, Yerba Maté leaves are used in an aqueous infusion dosage form in treatment of urinary tract and headaches. Formulations including Yerba Maté leaves are also used in treating mental and physical fatigue and are often included in weight loss programs as oral and topical supplementary agents. Pharmaceutical activity of Yerba Maté is ascribed to caffeine, caffeoylquinic acids, and caffeine-like polyphenols.

**[0007]** Similar weight-reduction activity is also exhibited by another plant - Guarana (*Paulinia cupana*, *P. sorbolis*) which contains large amounts of caffeine and other polyphenols - chlorogenic acids (CGA). According to Hurel, J.P., in 1993 (published French patent application 2 712 191 A1), caffeine included in Guarana extracts is the primary agent responsible for body weight decrease. However, use of Yerba Maté or Guarana as a single agent will result only in a momentary effect, and after finishing the treatment an immediate increase of body weight is observed.

[0008] U.S. Patent No. 5,804,596 discloses the activity of *Coleus Forskholii* root extract (ForsLean®). That extract contains the active agent forskolin (diterpene forskolin). The biological mechanism of diterpene forskolin activity is widely described in the medical literature, and many clinical evaluations have been performed. Those evaluations relate to different activities of diterpene forskolin, e.g., broncholytic (for treating bronchial asthma), relaxation of the arteries (for treating hypertension and cardiovascular system disorders), treating glaucoma, and impotence. Studies conducted on rats showed that low doses of diterpene forskolin are not effective. Only the use of very high doses resulted in distinct body weight reduction. However, in the case of humans such high doses would cause hypotensive effects and harmful high inotropic activity on heart muscle.

[0009] The pharmacological activity of birch leaves (*Betula Alba, betulae folium, Betula pendula Roth*) was not a subject of such extensive study as in the case of the herbs described above, but it was widely used in Europe for centuries. Birch leaves contain approx. 2-3 % of flavonoid glycosides, as well as triterpen alcohols and esters, previously regarded as saponines. Fresh birch leaves also include approx. 0.5 % of ascorbic acid. An aqueous extract of birch leaves is known as a mild diuretic agent and is used to irrigate the urinary tract, to remove sand, and to prevent inflammation of the urinary tract. It was also used orally.

[0010] Human obesity can be also treated with Orlistat® - ([2S-[2a(R\*),3b]]N-formyl-L-leucine 1-[(3-hexyl-4-oxo-2-oxetanyl)methyl]dodecyl ester), which is sold under the trademark Xenical® (U.S. Patent No. 4,598,089). A daily dose of that drug is three capsules containing 120 mg of Orlistat®. Inhibition of lipase activity induced by

Orlistat® means that 30% of consumed fat goes through the digestive tract without decomposition and is not absorbed. However, that drug exhibits some side effects such as fatigue, headaches, stomachaches, oily diarrheas, gases, and flatulence. It is not suitable for children and should not be used by pregnant and breast-feeding women.

[0011] An object of the invention is to provide a composition for treating human obesity, which would be effective in accelerating weight loss, and which would be characterized by the lack of major side effects and would help to maintain the lower body weight obtained during slimming.

#### SUMMARY OF THE INVENTION

[0012] Surprisingly, it has been found that a certain combination of different herbs and extracts of plant origin acts more effectively than each of the components alone. The components act in concert (synergistically) strengthening together the two main effects of the mixture, i.e., thermogenetic effects (increased metabolic rate) and a decrease of the absorption of fat.

[0013] According to the invention, a formulation for treating obesity and associated metabolic syndrome, comprising a combination of selected vegetable extracts, includes:

[0014] 20-90% wt. of Green tea extract, containing more than 70 % of catechines, preferably containing epigallocatechin galate (EGCG),

[0015] 2-30 % wt. of *Coleus forskholii* extract, containing at least 10 % of diterpene forskolin,

[0016] 5-58 % wt. of Yerba Maté extract, containing 2-4 % of caffeine and caffeoylquinic acids (CGA),

[0017] 7.5-45% wt. of *Betula alba* extract containing at most 3% of flavonides.

[0018] In another embodiment, Guarana extract is used instead of Yerba Maté extract. The following composition of the formulation was used:

[0019] 20-80% wt. of Green tea extract, containing more than 70 % of catechines, preferably containing epigallocatechin galate (EGCG),

[0020] 2-30 % wt. of *Coleus forskholii* extract, containing at least 10 % of diterpene forskolin,

[0021] 5-50% wt. of Guarana extract, containing more than 8% of caffeine and caffeine-like polyphenols (chlorogenic acids-CGA),

[0022] 7.5-45 % wt. of *Betula alba* extract containing at most 3% of flavonides.

[0023] The formulation of the invention can further include an effective amount of vegetable extract of white kidney beans (*Phaseolus Vulgaris*).

[0024] Preferably, Green tea extract is an extract obtained by water and/or ethyl acetate + water extraction at a low temperature and under reduced pressure.

[0025] In another preferred embodiment, Green tea extract is an extract obtained by alcohol extraction, or extraction conducted in the presence of fat solvents, for example selected from a group including: methanol-chloroform mixture, alcohol ethers and detergents, at a low temperature and under reduced pressure.

[0026] In one embodiment, Green tea extract includes at least 30 % of EGCG. In another embodiment, Green tea extract includes at least 50 % of EGCG, and in another one the Green tea extract includes at least 80 % of EGCG

[0027] Depending upon the dosage forms, the formulation of the invention can include non-active excipients or fillers selected from a group including: silicon dioxide, magnesium stearate, laurylsulphate, other surfactants, for example selected from a group including: sodium carboxymethylcellulose, hydroxypropylmethyl cellulose, and microcrystalline cellulose, anti-caking agents, such as dicalcium phosphate; and materials forming the shell of a capsule. According to in vitro and in vivo animal and human studies, the application of the formulation of the invention effectively accelerates weight loss.

[0028] One of the most important advantages of the formulation of the invention apart from the acceleration of weight loss is the prevention of weight regain after the termination of a slimming cure. The preparation also shows hypo-lipidemic and antihypertensive effects.

[0029] The formulation of the invention can be used in the form of infusions, teas, capsules, tablets, chewing gums, and powders that can be dissolved in water.

[0030] A daily dose of the formulation in the case of humans is equal to:

[0031] 250-2000 mg of Green tea extract,

[0032] 20-300 mg of *Betula alba* extract,

[0033] 30-400 mg of Yerba Maté or

[0034] 20-350 mg of Guarana,

[0035] 30-600 mg of *Coleus forskholii*.

[0036] In the case of the addition of white kidney bean extract in the formulation, the daily dose for humans would be 500-4000 mg of white kidney bean extract.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0037] The invention is illustrated by the examples given below, and its effects by the accompanying drawings in which:

[0038] Fig.1 shows a comparison between lipase inhibitory activity of the formulation of the invention and a Xenical® formulation.

[0039] Fig. 2A shows comparisons of the slowing down of the increases of body weight of growing rats after 12 weeks of diet supplemented with either the formulation of the invention or pure extracts of Yerba Maté, Guarana, *Coleus forskholii*, *Betula alba*, and Green tea (EGCG).

[0040] Fig 2B shows a comparison of the mean changes of the body weight of rats after 12 weeks of a diet supplemented with: either the formulation of the invention (XSIII) or pure extracts of Yerba Maté, Guarana, *Coleus forskholii*, *Betula alba* and Green tea (EGCG).

[0041] Fig. 3 shows comparisons between mean food intake in rats in groups treated with the formulation of the invention (XSIII) and pure extracts of Yerba Maté, Guarana, *Coleus forskholii*, *Betula alba*, Green tea (EGCG) after 12 weeks.

[0042] Fig.4 shows the effect of the formulation of the invention on resting metabolic rate on 12 healthy volunteers.

[0043] Fig. 5 shows the change of body weight (in %) during consecutive 14 weeks on 10 patients following a low calorie diet supplemented with the invention, in comparison with patients following the same low calorie diet and supplemented with a placebo (the study followed the rules of good clinical practice and was performed at a university hospital).

[0044] Fig. 6 shows the decrease of body fat weight (in kg) after 14 weeks of a low calorie diet supplemented with the formulation of the invention or a placebo.

[0045] Fig. 7 shows the decrease of LDL plasma cholesterol concentration (in mg/dl) after 14 weeks of a low calorie diet supplemented with the formulation of the invention or a placebo.

[0046] Fig. 8 shows the decrease of total plasma cholesterol concentration (in mg/dl) after 14 weeks of a low calorie diet supplemented with the formulation of the invention or a placebo.

[0047] Fig. 9 shows the decrease of total plasma triglycerides concentration (in mg/dl) after 14 weeks of a low calorie diet supplemented with the formulation of the invention or a placebo.

## DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0048] Example 1 Capsule composition

[0049] The formulation of the invention can be administered in the form of capsules. An example of a capsule composition is presented below.

[0050]	Green tea extract	325 mg,
[0051]	<i>Betula alba</i>	30 mg,
[0052]	Yerba Maté	30 mg,
[0053]	<i>Coleus forskholii</i>	10 mg,
[0054]	Total weight of active agents	395 mg,
[0055]	Silicon dioxide	12 mg,
[0056]	Magnesium stearate	5 mg,

[0057]	Lauryl sulphate	5 mg,
[0058]	Total weight of supplementary components	22 mg,
[0059]	Capsule shell	95 mg,
[0060]	Total weight of the capsule	512 mg.

[0061] Example 2 Capsule composition

[0062] Another example of a capsule composition is presented below.

[0063]	Green tea extract	325 mg,
[0064]	<i>Betula alba</i>	30 mg,
[0065]	Yerba Maté	30 mg,
[0066]	<i>Coleus forskholii</i>	10 mg,
[0067]	Total weight of active agents	395 mg,
[0068]	Silicon dioxide	12 mg,
[0069]	Magnesium stearate	15 mg,
[0070]	Total weight of supplementary components	17 mg,
[0071]	Capsule shell	95 mg,
[0072]	Total weight of the capsule	507 mg.

[0073] Example 3. Inhibiting lipase activity

[0074] Human obesity can be treated by reducing the digestion of fats and fat absorption. Fats must be decomposed by lipase before they are absorbed by the organism. Inhibiting lipase activity causes a considerable reduction of fat absorption,

which decreases calorie intake. Such a mechanism illustrates the activity of Roche's anti-obesity formulation Xenical® (Orlistat®).

[0075] Fig. 1 shows the comparison between the in vitro effect of the innovation on the activity of pancreatic lipase, and various concentrations of Xenical® (0.5-100 mg) after 30, 45, and 60 minutes at a temperature of 37°C. The composition of the formulation of the invention is presented below in Table 1

Table 1

Component	% (wt.)
Green tea extract	82.3
<i>Coleus forskholii</i> extract	2.5
Yerba Maté extract	7.6
<i>Betula alba</i> extract	7.6

[0076] In the case of the formulation of the invention, lipase activity was already reduced after 30 minutes. An increase of the time period to 45 and 60 minutes resulted in larger reduction of lipase activity in the case of lower concentrations (down to 30 mg) of the formulation of the invention. Maximum reduction of lipase activity obtained was equal to approx 80 %. Although the Xenical® (Orlistat®) inhibits lipase activity stronger than the formulation of the invention, and at a concentration of 10 mg after 45 and 60 minutes causes complete inhibition of lipase activity, it leads also to negative side effects described herein.

[0077] Example 4. Effect of the formulation of the invention and its components on reduction of body weight increase in rats.

[0078] The study was conducted to prove the influence of the formulation of the invention (the composition shown in Table 1) on the weight of laboratory rats in comparison to the groups fed with separate ingredients of the formulation.

[0079] 72 female Wistar rats (initial weight 173-209 g) were bred to the age of 8 weeks and housed in groups of 6 rats in one cage for 7 days (12h/12h light/dark cycle) in temperature and humidity controlled conditions. After 7 days of adaptation, healthy rats selected for the experiment were divided into 6 groups, each receiving one of the following substances: the formulation of the invention, *Coleus forskholii*, *Betula Alba*, Yerba Maté, Green Tea Extract (EGCG), and Guarana. The substances were administered orally (by gavage) in the form of solutions. During the experiment the animals were fed with ssniff® R (purchased from ssniff Spezialdiäten GmbH, Germany) - a certified pellet chow, and municipal water. The doses, which were calculated to correspond with the human daily doses, are presented in Table 2.

Table 2.

Substance	Daily dosage [mg]
Formulation of the invention	6.95
<i>Coleus forskholii</i>	0.17
<i>Betula Alba</i>	0.5
Yerba Maté	0.5
EGCG	5.33
Guarana	0.42

[0080] Body weights were measured and recorded once per week.

[0081] 12 weeks of observation of Wistar rats proved that the increase of body weight is at the lowest level in the group treated with the formulation of the invention. Values of the mean body weight increase in the tested groups are presented in Table 3 and in Fig. 2B.

Table 3

Group treated with	Mean body weight [g]
Formulation of the invention	59.8
<i>Colleus forskholii</i>	78.2
<i>Betula Alba</i>	76.7
Yerba Maté	71.0
EGCG	73.9
Guarana	68.7

[0082] The increase (%) of body weight in rats during the entire experiment are also presented in Fig. 2A.

[0083] Additionally, the mean amount of pellet-chow eaten by rats in appropriate group was measured. The results are presented in Fig. 3. It is clear that the mean food intake is lowest in the group treated with the formulation of the invention.

[0084] The experiment proved that the combination of the natural substances present in the formulation of the invention is effective in reducing body weight in rats, and acts much stronger than its individual ingredients.

[0085] Example 5. Effect of the formulation of the invention - study conducted on humans.

[0086] Advantageous properties of the formulation of the invention were confirmed by a study conducted on healthy volunteers. The aim of the study was to check the safety and efficacy of the invention and to examine the influence of the formulation of the invention when used as a food supplement on the resting metabolic rate. Mean resting metabolic rate increased in the treated group from  $4.3 \pm 0.2$  to  $4.8 \pm 0.2$  kJ/min after 3 days (Fig. 4), whereas the exercise metabolic rate remained unchanged (not shown).

[0087] Another human study – a double blind, placebo controlled clinical study - was performed under GCP (good clinical practice) to examine the decrease of body weight of patients on a low calorie diet (1000 kcal/day), body fat weight, increase of non-fat body weight (figure improvement), decrease of plasma LDL cholesterol, total cholesterol, and plasma triglycerides concentration. The study lasted 14 weeks. The composition of the formulation of the invention is presented below in Table 4:

Table 4

Component	% (wt.)
Green tea extract	79.5
<i>Coleus forskholii</i> extract	2.4
Guarana extract	6.2
<i>Betula alba</i> extract	11.9

[0088] The influence of the formulation of the invention was examined during tests performed on 48 obese patients divided into two groups who completed the study. Both groups received a low calorie diet (1000 kcal/day), but the diet of only one group was supplemented with the formulation of the invention ("treated group").

[0089] After 14 weeks, body weight decrease was equal to 6.06% in the control group and 9.28% in the treated group that was receiving the formulation of the invention (Fig. 5). Those values expressed in kilograms are equal to 5.54 kg and 8.51 kg, respectively. The difference between those two groups was 53.6 %. Differences were found to be statistically significant.

[0090] The decrease of body fat concentration was equal to -4.0 kg in the control group and -6.6 kg in the treated group (Fig. 6). Simultaneously, the decrease of LDL plasma cholesterol concentration was 1.58 mg/dl in the control group and was 6.08 mg/dl in the treated group (Fig. 7), and a decrease of total plasma cholesterol of 3.92 mg/dl and of 19.42 mg/dl (Fig. 8), respectively, was observed. The reduction of the cholesterol concentration was accompanied by a decrease in plasma triglyceride concentration of 6.63 mg/dl in the treated group, while in the control group the triglyceride concentration increased by 1.42 mg/dl (Fig. 9).

[0091] The quoted studies have verified:

[0092] that the formulation of the invention accelerates weight loss during dietary treatment of obesity.

[0093] that the formulation of the invention contains active components whose effects synergistically strengthen each other, so that the effect of their combination is significantly greater than the effect of any individual component alone.

What is claimed is: